

Atty. Dkt. No. P-6011
United States Serial No. 10/721,091

AMENDMENTS TO THE CLAIMS

Please amend the previously presented claims according to the changes indicated in the following claim listing.

Please withdraw claims 40-58. Claims 19-39 were previously withdrawn.

1. (currently amended) A method for quantifying an analyte in a sample, said method comprising:
 - a) administering a fusion protein to said sample, said fusion protein comprising a functional mutant periplasmic glucose-galactose binding protein ([[P]]GGBP), at least one labeling moiety and at least one fluorescent protein, wherein said fusion protein has a dissociation constant of at least 1 mM towards said analyte;
 - b) measuring the luminescence value of said fusion protein at a first emission wavelength when said analyte is not bound to said functional mutant periplasmic glucose-galactose binding protein;
 - c) measuring the luminescence value of said fusion protein at said first emission wavelength, when said analyte is bound to said functional mutant periplasmic glucose-galactose binding protein;
 - d) determining the difference [[in]] between the measured the luminescence value of (b) and the measured luminescence value of (c) at said first emission wavelength, wherein said difference [[in]] between said measured luminescence values at said first emission wavelength is due to resonance energy transfer between said labeling moiety and said fluorescent protein when said analyte is bound to said fusion protein;

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wherein said difference ||in|| between measured luminescence at said first emission wavelength is indicative of the amount of analyte in said sample.

2. (original) The method of claim 1, wherein said measuring is performed at more than one time point in the same sample.
3. (previously amended) The method of claim 2, wherein said measurement can be made continuously.
4. (original) The method of claim 1, wherein said fusion protein binds reversibly to said analyte.
5. (original) The method of claim 1, wherein said sample is a biological fluid.
6. (currently amended) The method of claim 1, wherein said ~~quantifying~~ determining the difference further comprises calculating a ratio of the ||said|| difference in (d) ||and|| ~~with a difference in the measured luminescence value of (b) and the measured luminescence value of (c) at a second wavelength~~ second value, wherein said second value is determined by (i) measuring the luminescence value of said fusion protein at a second emission wavelength when said analyte is not bound to said functional mutant periplasmic glucose-galactose binding protein; (ii) measuring the luminescence value of said fusion protein at a second emission wavelength when said analyte is bound to said functional mutant periplasmic glucose-galactose binding protein; and (iii) determining the difference between the measured luminescence value of (i) and the measured luminescence value of (ii) to determine said second value.

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7. (original) The method of claim 1, wherein said fusion protein comprises at least two fluorescent proteins.
8. (original) The method of claim 7, wherein said quantifying comprises calculating a ratio of the fluorescence of said at least two fluorescent proteins.
9. (original) The method of claim of claim 8, wherein said at least two fluorescent proteins are not identical.
10. (canceled)
11. (canceled)
12. (currently amended) The method of claim [[10]] 1, wherein said analyte is glucose.
13. (original) The method of claim 1, wherein said at least one fluorescent protein is selected from the group consisting of a green fluorescent protein (GFP), red-shifted GFP (rs-GFP), a red fluorescent protein (RFP), a yellow fluorescent protein (YFP), a cyan fluorescent protein (CFP), a blue fluorescent protein (BFP), enhanced versions thereof and mutations thereof.
14. (original) The method of claim 13, wherein said at least one fluorescent protein is RFP.
15. (original) The method of claim 14, wherein said RFP is selected from the group consisting of DsRed2, HcRed1, DsRed-Express and mutations thereof.
16. (previously amended) The method of claim 15, wherein said RFP is DsRed2(C119A).
17. (original) The method of claim 1, wherein said labeling moiety is a fluorophore.

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18. (previously amended) The method of claim 17, wherein said fluorophore is selected from the group consisting of fluorescein, acryoldan, rhodamine, eosin, pyrene, acridine orange, PyMPO, N-(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene-2-yl)iodoacetamide, N-(4,4-difluoro-5,7-diphenyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-N'-iodoacetylthylenediamine, and an Alexa dye.
19. (withdrawn) A composition comprising a fusion protein portion and at least one labeling moiety, said fusion protein portion comprising a functional periplasmic binding protein and at least one fluorescent protein.
20. (withdrawn) The composition of claim 19, wherein said functional periplasmic binding protein is selected from the group consisting of glucose-galactose binding protein (GGBP), maltose binding protein (MBP), ribose binding protein (RBP), arabinose binding protein (ABP), dipeptide binding protein (DIPBP), glutamate binding protein (GluBP), iron binding protein (FeBP), histidine binding protein (HBP), phosphate binding protein (PhosBP), glutamine binding protein, oligopeptide binding protein (OppA) and derivatives thereof.
21. (withdrawn) The composition of claim 20, wherein said functional BP is GGBP or a derivative thereof.
22. (withdrawn) The composition of claim 21, further comprising at least one additional fluorescent protein.
23. (withdrawn) The composition of claim 22, wherein said at least two fluorescent proteins are not identical.

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24. (withdrawn) The composition of claim 19, wherein said at least one fluorescent protein is selected from the group consisting of a green fluorescent protein (GFP), a red-shifted GFP (rs-GFP), a red fluorescent protein (RFP), a yellow fluorescent protein (YFP), a cyan fluorescent protein (CFP), a blue fluorescent protein (BFP), enhanced versions thereof, and mutations thereof.
25. (withdrawn) The composition of claim 24, wherein said at least one fluorescent protein is RFP.
26. (withdrawn) The composition of claim 25, wherein said RFP is selected from the group consisting of DsRed2, HcRed1, dsRed-Express and mutations thereof.
27. (withdrawn) The composition of claim 26, wherein said RFP is the DsRed2 mutant DsRed2(C119A).
28. (withdrawn) The composition of claim 27, wherein said labeling moiety is a fluorophore.
29. (withdrawn) The composition of claim 28, wherein said fluorophore is selected from the group consisting of fluorescein, acryoldan, rhodamine, BODIPY, acridine orange, eosin, pyrene, acridine orange, PyMPO, alexa fluor 488, alexa fluor 532, alexa fluor 546, alexa fluor 568, alexa fluor 594, alexa fluor 555, alexa fluor 633, alexa fluor 647, alexa fluor 660 and alexa fluor 680.
30. (withdrawn) A vector comprising a nucleic acid sequence coding for the fusion protein portion of the composition of claim 19.
31. (withdrawn) A host cell comprising the vector of claim 30.

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32. (withdrawn) A method of producing a protein, comprising culturing the host cell of claim 31 under conditions such that said protein is expressed, and recovering said protein.
33. (withdrawn) A kit for detecting the concentration of an analyte in a sample, said kit comprising the composition of claim 19.
34. (withdrawn) The kit of claim 33, wherein said functional periplasmic binding protein is selected from the group consisting of glucose-galactose binding protein (GGBP), maltose binding protein (MBP), ribose binding protein (RBP), arabinose binding protein (ABP), dipeptide binding protein (DIPBP), glutamate binding protein (GluBP), iron binding protein (IreBP), histidine binding protein (HBP), phosphate binding protein (PhosBP), glutamine binding protein, oligopeptide binding protein (OppA) and derivatives thereof.
35. (withdrawn) The kit of claim 34, wherein said functional periplasmic binding protein is glucose-galactose binding protein (GGBP) or a derivative thereof.
36. (withdrawn) The kit of claim 35, wherein said analyte is glucose.
37. (withdrawn) The kit of claim 36, wherein said at least one fluorescent protein is selected from the group consisting of a green fluorescent protein (GFP), a red-shifted GFP (rs-GFP), a red fluorescent protein (RFP), a yellow fluorescent protein (YFP), a cyan fluorescent protein (CFP), a blue fluorescent protein (BFP) and enhanced versions thereof.
38. (withdrawn) The kit of claim 37, wherein said at least one fluorescent protein is RFP.

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39. (withdrawn) The kit of claim 38, wherein said RFP is discosoma red fluorescent protein (DsRed2).
40. (withdrawn) A method for quantifying an analyte in a sample, said method comprising:
- a) administering a fusion protein to said sample, said fusion protein comprising a mutant glucose/galactose binding protein (GGBP), at least one labeling moiety and at least one fluorescent protein, wherein said mutant GGBP comprises at least one amino acid substitution selected from the group consisting of a cysteine at position 11, a cysteine at position 14, a cysteine at position 19, a cysteine at position 43, a cysteine at position 74, a cysteine at position 107, a cysteine at position 110, a cysteine at position 112, a cysteine at position 113, a cysteine at position 137, a cysteine at position 149, a cysteine at position 152, a cysteine at position 182, a cysteine at position 213, a serine at position 213, an arginine at position 213, a cysteine at position 216, a cysteine at 236, a cysteine at position 238, a serine at position 238, a cysteine at position 287, a cysteine at position 292 and a cysteine at position 296; and
 - b) measuring the luminescence of said fluorescent fusion protein, wherein said luminescence of said fluorescent fusion protein changes in response to the binding of said analyte to said mutant GGBP.
41. (withdrawn) The method of claim 40, wherein said measuring is performed at more than one time point in the same sample.
42. (withdrawn) The method of claim 40, wherein said measurement can be made continuously.

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43. (withdrawn) The method of claim 40, wherein said fusion protein binds reversibly to said analyte.
44. (withdrawn) The method of claim 40, wherein said sample is a biological fluid.
45. (withdrawn) The method of claim 40, wherein said fusion protein comprises at least two fluorescent proteins.
46. (withdrawn) The method of claim 45, wherein said quantifying comprises calculating a ratio of the fluorescence of said at least two fluorescent proteins.
47. (withdrawn) The method of claim of claim 46, wherein said at least two fluorescent proteins are not identical.
48. (withdrawn) The method of claim 40, wherein said functional PBP is selected from the group consisting of glucose-galactose binding protein (GGBP), maltose binding protein (MBP), ribose binding protein (RBP), arabinose binding protein (ABP), dipeptide binding protein (DPBP), glutamate binding protein (GluBP), iron binding protein (FeBP), histidine binding protein (HBP), phosphate binding protein (PhosBP), glutamine binding protein (GIBP), oligopeptide binding protein (OppA) and derivatives thereof.
49. (withdrawn) The method of claim 48, wherein said functional PBP is GGBP or a derivative thereof.
50. (withdrawn) The method of claim 48, wherein said analyte is glucose.
51. (withdrawn) The method of claim 40, wherein said at least one fluorescent protein is selected from the group consisting of a green fluorescent protein (GFP), red-shifted GFP

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(rs-GFP), a red fluorescent protein (RFP), a yellow fluorescent protein (YFP), a cyan fluorescent protein (CFP), a blue fluorescent protein (BFP), enhanced versions thereof and mutations thereof.

52. (withdrawn) The method of claim 51, wherein said at least one fluorescent protein is RFP.
53. (withdrawn) The method of claim 52, wherein said RFP is selected from the group consisting of DsRed2, HcRed1, DsRed-Express and mutations thereof.
54. (withdrawn) The method of claim 53, wherein said RFP is the DsRed2 mutant DsRed2(C119A).
55. (withdrawn) The method of claim 40, wherein said labeling moiety is a fluorophore.
56. (withdrawn) The method of claim 55, wherein said fluorophore is selected from the group consisting of fluorescein, acryoldan, rhodamine, eosin, pyrene, acridine orange, PyMPO, N-(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene-2-yl)iodoacetamide, N-(4,4-difluoro-5,7-diphenyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-N'-iodoacetylthylenediamine, and an Alexa dye.
57. (withdrawn) The method of claim 1, wherein said fluorescent protein is the acceptor for said resonance energy transfer.
58. (withdrawn) The method of claim 1, wherein said labeling moiety is the acceptor for said resonance energy transfer.